“TO STUDY THE GENETIC DIVERSITY AND COMPARITIVE PHYTOCHEMICAL ANALYSIS IN RHEUM SPECIES”

SYNOPSIS SUBMITTED
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Background

The importance and use of high-altitude medicinal plants in general/traditional healthcare system, provides clue to new frontier areas of research in plant biodiversity conservation is a well-established feature (Li *et al*., 1997). The present research proposal is focused to look in depth the genetic and molecular difference of the medicinal plant material that are used in state of Uttarakhand by local population for the various ailments. In the priority area of conservation of plant resources, preservation and maintenance of medicinal plants in the wild and their systematic cultivation outside their natural habitat of growth has assumed significance, especially, during the last twenty years, because of their rate of depletion due to various reasons which are detrimental to the natural revival/ growth of these species. Biodiversity analysis, phytochemical investigations and germ-plasm conservation of these plants often go side by side. In, India, a country lying in tropical zone is a cradle for many species of medicinal plant. Moreover, like other developing nations, bio-diversity of medicinal plants in India is in perilous state - due to interminable capitalization of these medicinal plants by pharma companies.

The plant biotechnology offers alternative to conventional plant improvement techniques. Many opportunities for using biotechnology in plant breeding have been identified, most of which have application to the urgent problems of the improvement of forest crop in developing countries.

Rheum species is a perennial herbaceous plant growing with fleshy roots. The plant have upright growing stem and basal, deciduous leaves extending from short, broad rhizomes having deciduous core. The arrangement of flowers on a stalk are terminal and loose branching clusters-like pedicels. The flowers are hermaphrodite consisting whitish to pinkish green, campanulas and hairless sepals and petals (six tepals). Many of the species *Rheum* have nutraceutical uses originated in Asia almost 2,000 years ago. All plant parts contain the poison oxalic acid, though its concentration in parts used for preparation of food is very low especially leaf stem and petioles. The tart flavour of the plant is due to the nontoxic component, malic acid. The Rheum plant also produce some other poisonous compounds, like citric acid & anthraquinone based glycosides. The raw as well as cooked leaf blades are poisonous to animals as well as to humans, if consumed in large amounts. Rheum species can also be propagated by seeds and cutting up the crowns of large plants.

An interpretation of genetic difference in condition, amount and pattern is of prime importance in study of genetics. This fundamental knowledge is a basis of tests involving evolutionary hypothesis, but also of immense importance for phytochemical profiling, *in vitro* techniques for cultivation of effective germ-plasm upgradation and *ex situ* plant conservation & preservation strategies (Dong and Wagner, 1994) and authentication of the selected stands and clonal plant material.

India with its immense biotic wealth, has more than 7000 species reportedly used for food and medicinal purposes. Most of these plants are being over exploited in its natural habitat for the extraction of drugs. It will be wise to study about these species of local/indigenous medicinal/nutraceutical/commercially viable plants at their genetic and molecular levels along with the development of efficient management and conservation strategies to study genetic & phytochemical diversity. Altitudinal variation at genetic and phytochemical level can be exploited to select the different useful genotypes as cultivars to avoid variation in batch-to-batch extraction of drugs (Prasad and Purohit, 2001). Globally, emphasis is laid on exploitation of natural resources and patenting of plants in developing as well as developed countries to generate databases on indigenous medicinal/nutraceutical and commercially valuable plants which can be used further for future reference.

Unplanned development and mismanagement of ecosystem led to over exploitation of medicinal and commercially important plants from scientifically non-managed natural resources. This not only resulted in shortage of various medicinal and commercially important herbs but also leads to the extinction of several species in their natural habitat. In order to meet the increasing demand from industries for these plants, it becomes even more important to conserve these medicinal plant species by domestication/cultivation or adopting different *ex-situ* and *in situ* conservation strategies for their sustainable development. Emphasis is also laid on cultivation of plants of wild form, instead of collection from wild would ensure their proper botanical identity, genetic and molecular improvement, quality of plant and continuity of supply. These medicinal and nutraceutically important plants in traditional and local healthcare practices provides valuable clues for new areas of research and biodiversity conservation strategies. The present research study aimed to look into the genetic and phytochemical investigation of Rheum plant that is used by local people for commercial purpose or for curing various ailments.

In the priority area of natural resource conservation and preservation of plants in the wild and their systematic cultivation outside their natural habitat is gaining significant importance during the last two decades, because of the alarming rate of depletion of these plant resources inimical to the natural regeneration of species. Biodiversity conservation strategies and germ-plasm methodology are of immense importance in plant conservation and breeding programs (Rokaya *et al*., 2012).

Plant Description

Rheum, genus belongs to Polygonaceae family, which is more commonly known as Rhubarb. This genus report to have of 60 species globally, out of which, North Western Himalayan region of India harbour 10 species. *Rheum* sp. is a continually recurring herbaceous plant growing with fleshy roots. The plant have upright growing stem and basal, deciduous leaves extending from short, broad rhizomes having deciduous core. The arrangement of flowers on a stalk are terminal and loose branching clusters-like pedicels. The flowers are hermaphrodite consisting whitish to pinkish green, campanulas and hairless sepals and petals, 6–9 pollen containing anther and filaments, distinct compact stigma and inclined ovary (Dhiman, 2004). Rheum have (x = 11) chromosomes. Many species of Rheum exhibit ploidy levels i.e. diploid, tetra-ploid &hexa-ploid (Chin & Youngken, 1947; Edman, 1929; Englund, 1983; Gilmore et al., 2014; Foust & Marshall, 1991; Liu et al., 2010; Morse, 1901; Turner, 1938). Rheum sp. generally found in temperate and sub-tropical region of Asian continent at the altitude of 2800–3800 m (Kuhl and DeBoer, 2008; Nautiyal et al., 2003). In North-West Himalayan region of India, it is distributed from Jammu & Kashmir to the state of Uttarakhand (Nautiyal et al., 2003). Though, the majority of wild population is confined to Jammu & Kashmir Himalayan region.

Rheum, genus used in numerous traditional medicine system of China and Tibet for the treatment and prevention of piles, gastroenteritis, haemorrhage, kidney related diseases (nephritis), and different diseases leading to inflammation (Peigen et al., 1984). Indian medicine system focuses on utilization of plant roots for the treatment of cuts, bruises, wounds & bowel problems (Dhiman, 2004). Further, rhizomes of Rheum is used as a potent laxative, stimulant, antioxidant and preparation of general tonic for dyspepsia, ulcers, rheumatic pains (Agarwal, 1986; Farooq et al., 2014; Kaur et al., 2012). Foliage part of Rheum are also consumed in raw form as salads and cooked vegetables (Parveen and Wani, 2013; Treuren et al., 2011). Rhizomes of Rheum sp. is a good source of red dye for colouring the silk and woollen clothings (Debaish and Bhattacharya,

2008). According to the cited facts and literature about the immense importance of the plant species, still till date no study were carried out on genetic and molecular diversity & population structure arrangement and composition of plant at the molecular level in State of Uttarakhand. This type of study is an urgent requirement of time for planning, designing and executing conservation and management strategies for the plant in their natural habitat. Further, it will help in carefully choosing the varied germ-plasm for cultivation, maintenance and improvement of plant resource in near future for taxonomic implications.

Hypothesis

India, a country lying in a tropical zone is a cradle to many medicinal/nutraceutical and commercially viable plant species. Moreover, like other developing nations, bio-diversity of medicinal plants in India is in perilous state - due to interminable capitalization of these medicinal plants by pharma companies. The hypothesis of the proposed problem involve studies on available material of various populations of *Rheum sp.* will be evaluated through molecular markers for the assessment of genetic variability and diversity with respect to the active components in its altitudinal zone of distribution. The conventional approach may take several years to yield this information. It is proposed to standardize and isolate genomic DNA from the individuals of different populations. Standardize PCR conditions for the multiplication of the specific segment/region of the genes that could generate the database of the species in regard to the molecular diversity that could be used for recommending the most diverse source for the plant breeding programs. Thus, there is an urgent need to counter and tackle the problems like volume of genetic difference available in natural population of Rheum species in their habitat (Ma et al., 2014) and distribution of this genetic and molecular variation in different geographical zones i.e. natural habitat of Rheum sp. in Uttarakhand Himalaya.

National & International Status

A lot of molecular marker techniques are available for the evaluation of genetic and molecular diversity in geographical zone of distribution of population. DNA marker techniques such as Inter-Simple Sequence Repeats (ISSR), Randomly Amplified Polymorphic DNA, SSR and AFLP are frequently used for genetic analysis (Kishore et al., 2012; 2013; Tanhuanpäa et al., 2019; Tautz, 1989; Williams et al., 1990; Wang et al., 2012a; 2012b; 2014; Vos et al., 1995; Zietkiewicz et al., 1994).

The polymerase chain reaction (PCR) has revolutionized the way of obtaining genetic information (Mullis *et al.* 1986) through obtaining RFLP patterns in a specific segment of gene. Large amounts of DNA polymorphism can be detected using RAPD (Random Amplified Polymorphic DNA) technology that involves the amplification of anonymous DNA sequences using short primers of arbitrary nucleic sequence (Williams *et al*. 1990). With care, large numbers of RAPD markers are readily resolved (Grattapaglia and Sederoff, 1994, Plomion *et al*. 1995; Ranjan et al., 2010), resulting in a virtually unlimited supply of neutral markers. The random amplified polymorphic DNA techniques (RAPDs) have been shown to be effective in identifying useful polymorphism in both repetitive DNA and low copy DNA sequences (Williams *et al.* 1990). With both the methods, the somaclonal variation existing among the clonally propagated material can be detected and subsequently the variant clonal material if any can be eliminated from the batch.

The use of DNA based molecular markers in plant molecular biology research is to evaluate genetic and molecular diversity in plants is an important tool to study them in the zone of distribution of plant population of different species (Abdelmigid 2012; Agostini et al. 2008; Gajera et al. 2010; Liu et al. 2006;). These studies considered as an integration of the database generated from plant genetic studies and ecological data of the habitat to formulate productive plant conservation & preservation methods for the development of systematic well organized plant breeding programs. Recently developed, ISSR molecular technique employed to analyze difference in the various microsatellite regions spread across the plant genomes. They characterize individual gene loci more effectively than molecular approaches. ISSR markers primarily amplified by di, tri, tetra, or penta, 1-3 nucleotides which are randomly repeated (Zietkiewicz et al. 1994). Reproducibility for ISSR fingerprints is due to larger SSR based primers enabling rigorous standards of performance of amplified DNA. DNA fingerprinting by ISSR technique has

few drawbacks due to dominant markers. Then also, it is an important tool in dealing plant systematics & population genetics studies (Reddy et al., 2002). This technique is most commonly used for the assessment and analysis of diversity of plant population genetics & structures. (Baloch et al. 2010; Kishore et al., 2013; He et al. 2006;; Meloni et al., 2006; Manimekalai and Nagarajan 2006; Singh et al. 2011; Tabin et al., 2016). The organic acid components present in Rheum spp have also been analyzed using ion moderated partitioning chromatography, the HPLC technique that have been profoundly used in analysis. (Kimmo et al., 1999).

DNA based molecular markers analysis and assessment assist geneticists to monitor the precise distinction in gene sequences in different genotypes at a given different times. The strategy is of immense importance to the plant breeders to evaluate the genetic combinations of characters selected for breeding strategies over the generations giving an insight for a genetic belonging to a specific taxon for DNA marker based selection in future (Christiansen et al., 2002). Moreover, the information help the breeders in promoting functional genetic variation by incorporating in genetic pool of plants by alleles linked markers to gene loci, controlling quality traits. A greater range of variation was shown by the microsatellite loci with more numbers of repeated base sequence as compared to microsatellite loci with less number of repeated base sequences as shown in different crops like barley, buckwheat, maize, sea buckthorn, tomato, horse gram (He et al., 2003; Kishore et al., 2012; 2013; Ramsay et al., 2000; Sharopova et al., 2002; Sharma et al., 2013; 2015; 2018).

Objectives

The objectives of the research proposal are:

- **To analyse the effect of microbial/chemical elicitors on the structure and yield of the active constituent.**
- **Standardization of DNA isolation, purification, optimization of PCR conditions for the specific gene regions with the objective of screening various populations of *Rheum sp.* for polymorphism and genetic analysis.**
- **To document and analyse patterns of genetic variability and diversity within and among populations of *Rheum sp.* with the help of molecular genetic markers (DNA).**

Methodology of the proposed study

Year I

1. Collection of plant material
2. Screening and characterization of active phyto-constitutent. (Prasad and Purohit, 2001)
3. Isolation of genomic DNA from various individuals belonging to different populations of *Rheum sp.* and their storage (Xu-Mei Wang, 2010)
4. Standardization of components of Polymerase Chain Reaction like annealing temperature, Mg concentration, Taq polymerase, extension time etc. to amplify variable regions of nuclear and organelle genes. (Mullis et al, 1986)

Year II

1. Amplification of various genes/gene regions in different individuals and populations of *Rheum sp.* through PCR profiles, gel purification and their screening through ISSR/SSR analysis. (Xu-Mei Wang, 2010)
2. Scoring and recording of the polymorphism and preparation of spread sheet for their analysis.

Year III

1. If required the polymorphic regions will be sequenced.
2. Scoring and recording of polymorphic loci and their genetic analysis. (Gilmore et al, 2014)
3. Scoring of polymorphism, and analysis for variability and phylogeny.
4. **Statistical analysis:** In all experiments, triplets will be used and the collected data are subjected to Analysis of Molecular Variance (AMOVA),

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